

Laboratory of Pharmacology: Summary Statement

by Larry Hart

The Laboratory of Pharmacology initiates and carries out research programs that are primarily concerned with (1) mechanisms by which environmental pollutants and effectors cause toxicity, (2) the development of better test systems for detecting and evaluating early toxic effects of chemicals occurring as environmental contaminants, and (3) extrapolating from animal test systems across species and to man. The Laboratory is organized into three formal sections and one work group. The sections are Toxication-Detoxication, Marine Pharmacology and Biomedicine, and Pharmacokinetics; the work group is Molecular Pharmacology and Biomedicine.

Program

Toxication-Detoxication Section

This program area is concerned with the role of chemical metabolism in the production of tissue damage and toxicity. Metabolism leading to increased toxicity may be balanced by reactions with detoxifying systems. This balance between toxication and detoxication pathways may be altered by pollutants, other chemicals, pathologic states, physiological changes (age or pregnancy), species and strain differences, hormonal changes, or it may be different in one tissue from another. Chemical-metabolizing systems have been or are being characterized in tissues most likely to contact the environment, i.e., skin, intestine, lung, and liver, as well as in steroidogenic tissues containing germinal cells (ovary, testis). Characterization includes substrate and inhibitor specificity, ability to be activated, induced, or inhibited by exposure to other chemicals and changes in physiological status (e.g., pregnancy), and measurement and purification of multicomponent systems or enzyme families (e.g., cytochrome P-450-dependent mixed-function oxidase [MFO] systems or the glutathione *S*-transferases).

Two different forms of cytochrome: P-450 (absorption maxima of reduced-CO bound cytochrome occurred at 425.5 nm and 450 nm, respectively) were separated from the same rabbit lung microsomal preparation. The lipid requirement for maximal MFO activity is being studied in reconstituted systems. Cytochrome P-448 was purified from livers of control and 3-methylcholanthrene-pretreated rabbits; the cytochromes were identical based on purification procedures, monomeric molecular weight, and immunological tests.

Techniques are being developed to separate and culture lung cells for studying chemical toxication and detoxication mechanisms at the cellular level.

Mammalian skin has very low, but detectable, MFO activity. In mice, at least, this activity seems to be increased following exposure to shortwave ultraviolet irradiation (254 nm) or to a sunlamp—conditions that might mimic prolonged exposure to sunlight.

The effects of pretreating mice, rats, guinea pigs, and rabbits, with known inducers of hepatic MFO activity, on intestinal xenobiotic-metabolizing enzyme activities were studied. The induction of intestinal MFO enzymes varied with the animal species and the xenobiotic used as substrate, which is consistent with the idea that chemically induced toxicity in this tissue may be partially dependent on species.

The formation and subsequent biotransformation of chemically reactive alkene and arene oxides, formed from alkenes and polycyclic aromatic compounds, are being studied in liver and several extrahepatic tissues of rats and rabbits. Comparative studies utilize partially purified enzymes, subcellular fractions of tissue homogenates and isolated perfused lung, liver, and testis preparations. The relative importance of glutathione *S*-transferase and epoxide hydrase activities in the metabolism and detoxication of chemically different epoxides is being assessed. Substrate specificities with different synthetic oxides that are thought to be car-

cinogens and/or mutagens are tested. The effect of pre-exposure of animals to other xenobiotics, to depiction of required endogenous cofactors, and to normal physiological control mechanisms on enzyme activities and epoxide-induced toxicity is also determined.

All of the studies in this section seek to understand the relationships between xenobiotic metabolism and the localized toxicity (i.e., target organ), both acute and chronic, caused by exposure to environmental pollutants.

Marine Pharmacology and Biomedicine

This program area conducts studies on uptake, distribution, metabolism, and excretion of pollutants by various marine species, the role of metabolism of these pollutants in their storage and the chemical moiety stored in these species, and the use of marine organisms to identify those physiological processes that are especially sensitive to exposure to certain xenobiotics (e.g., organochlorines).

One area of major emphasis is the potential accumulation of carcinogens, mutagens, teratogens, cytotoxins, and marine toxins (i.e., chemicals that may be particularly dangerous to man) in aquatic species. The effect of exposure to pollutant mixtures on accumulation of chemicals is also studied.

Operation of a year-round facility (the C. V. Whitney Marine Laboratory, University of Florida, St. Augustine) allows investigations which expand and complement our seasonal (summer) studies at Mount Desert Island Biological Laboratory, Maine. Comparisons are being made of the effect of environmental temperature on metabolic, excretory, and storage processes for pollutants. Currently, the disposition of aliphatic and aromatic constituents of crude and refined oils is under investigation.

The biotransformation of various organic pollutants is studied in hepatic and extrahepatic tissues of vertebrate and invertebrate marine species from coastal Maine and Florida. Particular emphasis is placed on studying formation and further metabolism of chemically reactive electrophilic metabolites. Both cytochrome P-450-dependent microsomal mixed-function oxidases and alkene- or arene oxide-metabolizing enzymes (epoxide hydase and glutathione *S*-transferases) are being characterized in control marine species and in species pre-exposed to environmental contaminants such as the polycyclic aromatic hydrocarbons. Where biologically significant induction is observed, the induced system is being characterized in considerable detail, e.g., in little skates, where

cytochrome P-448 has been identified for the first time in an aquatic animal.

The pharmacokinetics of pollutant distribution in selected marine species is being determined and compared with mammals (in collaboration with the Pharmacokinetics Section). So far we have shown that a polychlorinated biphenyl (PCB) isomer is stored in different tissues in different marine species and that the biological half-life of PCB correlates with the ability of the species to metabolize it. Compared with mammalian systems, marine species have a relative deficit in the capability to carry out oxidative chemical metabolisms, while possessing adequate levels of enzymes for conjugation reactions. Thus, we have shown that water-soluble pollutants, such as 2,4-D and 2,4,5-T, are rapidly conjugated with amino acids and excreted in the bile and urine of teleosts and elasmobranchs.

Other areas being investigated include the importance of renal and hepatic organic anion transport systems in elimination of xenobiotic metabolites by marine species, the role of intracellular binding proteins (the glutathione *S*-transferases) in cellular transport and toxicity of organic anions, and the role of membrane function in relationship to target organ accumulation of xenobiotics or their metabolites and toxicity. Emphasis is placed on correlating *in vitro* biochemical and physiological measurements with *in vivo* function (e.g., osmoregulation). Since many endogenous compounds utilize active transport systems in various tissues of the body, these studies also attempt to elucidate interactions between xenobiotic metabolites and endogenous molecules utilizing the same transport mechanisms.

The Section is also involved in developing a national program in marine pharmacology and biomedicine as directly applicable to human health problems (models for human disease, sources of toxic materials for man). The main direct concern to the NIEHS will be to provide a focus for DHEW activities in marine biomedical studies.

Molecular Pharmacology and Biochemistry Work Group

This group is involved in studies which can be categorized as pharmacological and biochemical research on synthesis, metabolism, release, isolation, and identification of endogenous chemicals (lipids, proteins, enzymes) involved in lung function, both in normal and in disease states. Information is being used to develop markers for early human disease states of possible environmental origin.

One group is concerned with the identification and characterization of components which are lung specific or pulmonary disease-related which could

be used as diagnostic markers for pulmonary damage or disease. Current attention is being focused on: (1) the soluble alkaline phosphatases of the normal lung, (2) abnormal hydrolases present in pulmonary lavage effluents from patients with alveolar proteinosis, and (3) enzymic components of human amniotic fluid which originate in the fetal lung.

Human lung was found to contain two distinct alkaline phosphatases having molecular weights of 213,000 and 125,000 daltons. These enzymes appear to be lung-specific with the lower molecular weight form present in extracellular lining of airways. This work was done in collaboration with Duke University and George Washington University. Further studies have characterized the high molecular weight ($> 20 \times 10^6$ daltons) soluble alkaline phosphatase (AP) complex found in pulmonary lavage effluents of patients with alveolar proteinosis, a disease of suspected environmental etiology. After appropriate chemical treatment of the complex, a low molecular weight AP is released which appears similar to the AP's found in normal lung.

Another group of investigators is focusing on the potential of normal and neoplastic cells of the lung to synthesize polypeptide hormones. A pituitary-like cell has been identified in the lungs of several species and designated P cell. These cells may be target progenitors for lung tumors, such as oat cell carcinoma, which are capable of producing bioactive polypeptide hormones. Four mammalian squamous cell carcinomas were found to contain immunoreactive adrenocorticotrophic hormone (ACTH) and this immunoreactivity was associated with "big ACTH" rather than "small ACTH." "Big ACTH" was also found in the extract of bovine pituitary gland.

Since human bronchogenic squamous cell carcinomas are known to produce a large form of ACTH, an investigation has been initiated to purify this material from bovine pituitary glands so that a specific radioimmunoassay might be developed. Hypersecretion of cell-specific or tumor-specific polypeptides or proteins could serve as a means for sensitive detection of pulmonary neoplasia or pre-neoplasia.

A third group of investigators is studying the biosynthesis, release, and metabolism of prostaglandins (PG) and thromboxanes (TX) in pulmonary tissue. A transport system responsible for removing PG from the circulation into the lung was observed and characterized by the group. The structural requirement necessary for transport was examined using the isolated perfused rat lung. Some chemicals and anti-inflammatory agents inhibited the transport system. Exposure of rats to pollutants

(O₂, NO₂) inhibited the pulmonary metabolism of PG but did effect the transport system. The major metabolites of PG, 15-keto PG were found to be further metabolized by glutathione S-transferase to the corresponding glutathione conjugate.

PG and TX biosynthesis were studied. During the formation of PG and TX, a metabolite(s) of arachidonic acid (AA), the precursor substance for PG, was covalently bound to tissue protein. Since AA or the known end-products, PG and TX, did not react with tissue, reactive intermediates were probably formed which reacted with tissue. The nature of the reactive intermediate is currently under investigation. The covalent binding of these endogenous chemicals may be associated with or be responsible for pulmonary toxicity induced by environmental agents. Irritation of pulmonary tissue by exposure to pollutants initiates the biosynthesis and release of PG and TX. Covalent binding of AA metabolites was found in other tissues including platelets, seminal vesicles, kidney, and stomach.

These investigators also studied the co-oxygenation of chemicals during the formation of PG and TX. During this formation in guinea pig lung, benzpyrene (BP) was oxidized to metabolites which appeared to be quinones with little or no formation of monohydroxy- and dihydrodiol metabolites, but in the presence of NADPH, the major benzpyrene metabolite was dihydrodiol. Approximately 20% of the BP oxidized during the formation of PG was covalently bound to protein while only 5-8% of the metabolites formed in the presence of NADPH was bound to protein.

Activation of benzpyrene during the formation of PG and TX may be related to the induction of pulmonary carcinogenesis and may represent an alternate mechanism to oxidation of chemicals by the cytochrome P-450 system.

Pharmacokinetics Section

This section is conducting research in at least two major categories: (1) whole body distribution of pollutants and formulation of pharmacokinetic models for extrapolation, and (2) uptake, storage, metabolism, and release of pollutants and model compounds by isolated perfused organs, with present emphasis on lung and liver. The kinetics of chemical disposition in intact animals and isolated organs are being determined in order to construct mathematical models of these processes which can be used to better define and understand rate-limiting steps in the process, to extrapolate distribution profiles of chemicals from one tissue to another, one species to another and to man, and to predict tissue storage of pollutants from knowledge

of routes, dose, and number of exposures. Comparisons of the kinetics of pollutant distribution in mammalian vs. marine species are being carried out in collaboration with Marine Pharmacology and Biomedicine, as previously mentioned.

Through extensive collaboration with the Biomedical Engineering and Instrumentation Branch, DRS, NIH (Drs. R. L. Dedrick and R. Lutz), pharmacokinetic models have been developed for four PCB isomers in the rat. The biochemical and physiological parameters used in these models were determined from studies in bile-cannulated animals, isolated perfused liver studies, and whole animal disposition studies.

These models can predict the disposition of the PCBs after a single intravenous or oral dose. Recently the models have successfully predicted the disposition of PCBs in rats receiving multiple doses for six weeks. The pharmacokinetic models developed for rats were modified to enable extrapolation to the mouse. Model parameters such as tissue values, flow rates, and gut transport were adjusted in accordance with animal weight and the predicted PCB disposition compared to experimental data. Tissue and excreta concentration-time curves obtained in experimental animals generally agreed with model predictions. The species independence of the pharmacokinetic model will be tested in dog and monkey. Experimental data will be obtained from a contract with the University of Arizona.

The disposition of two additional PCB isomers (3,3',5,5'-PCB and 2,2',3,3',6,6'-PCB) was studied in rat to further examine the influence of degree and position of chlorination on disposition of PCB. The 4-CB, which does not have adjacent unsubstituted carbons, was metabolized more slowly than predicted from the degree of chlorination. The 6-CB, which has an adjacent unsubstituted carbon, was metabolized as rapidly as the 1-CB previously studied. The metabolites of various PCBs were isolated and identified as monohydroxy- and dihydroxydiol metabolites. Metabolism of PCBs appears to be a prerequisite for excretion. PCBs that are persistent in the body are those isomers that are slowly metabolized by animals.

The disposition of the major component of Firemaster BP-6 (2,4,5,2',4',5'-hexabromobiphenyl) was studied in rats. The polybrominated biphenyl appears to have similar pharmacokinetic properties to the corresponding hexachlorobiphenyl and the half-life of the hexabromo isomer was longer than the corresponding hexachlorobiphenyl isomer. A pharmacokinetic model for hexabromobiphenyl is being developed.

The disposition of Kepone, a halogenated insecticide, was also studied. Kepone was stored primar-

ily in the liver and excreted in the bile. The measured half-life of 18 days was due primarily to reabsorption of Kepone from the gut after excretion in the bile.

Another continuing major effort of the Section is in examination of pharmacokinetic functions of the lung with regard to both exogenous and endogenous chemicals. These studies involve the use of the isolated perfused rat, guinea pig, and rabbit lung preparations as research tools. With regard to exogenous chemicals, detailed models have been developed for the dynamic interaction of the perfused lung with circulating chemicals. With regard to endogenous chemicals, a pharmacokinetic model was developed for the prostaglandin detoxification system. From this model, it was evident that flow rates are important in examining transport systems in isolated organs. In general, elucidation of the rates and mechanisms of lung uptake, storage, and metabolism of chemicals may allow prediction of possible toxic interactions between environmental pollutants and other chemicals, including drugs and endogenous chemicals such as prostaglandins.

The major effort of the year was devoted to modification of existing perfused lung techniques and development of new methods to examine the uptake and metabolism of inhaled environmental pollutants. Several environmental carcinogens, e.g., benzo(a)pyrene, have been selected for study and attempts will be made to correlate the uptake and metabolism of these chemicals to the development of lung tumors.

Personnel

Two major personnel shifts took place during the year. Dr. L. G. Hart (Acting Laboratory Chief) moved into the position of Assistant to the Scientific Director, NIEHS, about midyear, and Dr. J. R. Fouts (Scientific Director) assumed the responsibility of Acting Chief. A group headed by G.E.R. Hook transferred from the Molecular Pharmacology and Biochemistry Work Group into the Laboratory of Environmental Toxicology later in the year. Other members of the group include Dr. J. W. Spalding (Research Biologist), Dr. D. Nadeau (Visiting Fellow), Dr. D. Bell (Guest Worker), and Ms. L. Gilmore (Biologist).

Additions to the laboratory were: Research Physiologist, J. B. Pritchard (with Dr. Bend); Research Chemist, L. Lazarus (with Dr. DiAugustine); Research Biologist, J. W. Spalding (transferred from LET, with Dr. Hook); Staff Fellow, W. Colburn (with Dr. Eling); Visiting Associates, A. Wilson (with Dr. Eling), H. Mukhtar, T. El-mamlouk (with Dr. Bend); Visiting Fellows,

O. Hernandez, R. Weatherby (with Dr. Bend), M. Szutowski, C. R. Wolf (with Dr. Philpot); D. Crutchley, A. Kung (with Dr. Eling), L. Ball (with Dr. Chhabra), M. Khan (with Dr. DiAugustine), J. Leakey (with Dr. Fouts); NIH Postdoctoral Fellow, J. Maguire (with Dr. Bend); Intergovernmental Personnel Act, G. Neufeld (with Dr. Bend), D. Bell (with Dr. Hook).

Losses from the Laboratory included: J. Maguire (NIH Postdoctoral Fellow), M. Szutowski (Visiting Fellow), O. Hernandez (Visiting Fellow, transferred to EBCB), M. Reasor (NIH Postdoctoral Fellow), J. Brittan (technician).

Other Activities

Dr. J. R. Bend: Member, Editorial Advisory Board for DRUG METABOLISM AND DISPOSITION; Visiting Scientist, C. V. Whitney Marine Laboratory, University of Florida, St. Augustine; Trustee, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine; Member, Committee on Environmental Pharmacology, American Society for Pharmacology and Experimental Therapeutics; invited participant in a symposium on Formation of Chemically Reactive Metabolites As a Cause of Drug Toxicity, sponsored by ASPET, New Orleans, La., 1976; invited participant at a conference on Aquatic Pollutants and Biological Effects with Emphasis on Neoplasia, sponsored by the New York Academy of Sciences, Sept. 1976; invited speaker at a symposium on Status of Predictive Tools in Application to Safety Evaluation: Present and Future, sponsored by NIH and NCTR, Little Rock, Ark., 1976; invited speaker on Metabolic Reactions *In Vivo* and *In Vitro* in conference on Short Term Tests for Carcinogenicity, sponsored by Toxicology Forum, Hunt Valley, Md., Feb. 1977; invited lecturer at the NATO Workshop on Ecotoxicology, University of Surrey, Guildford, England, July 11–August 5, 1977; invited speaker at symposium entitled Environmental Pharmacology of Lung, sponsored by ASPET, Columbus, Ohio, Aug. 1977; presented

seminar entitled Metabolism of Xenobiotics by Marine Species, Department of Pharmacology, University of Wisconsin, Dec. 1976.

Dr. R. S. Chhabra: Member of the Library Committee, NIEHS; invited speaker to International Symposium on Nutrition Drug Interactions, Ames Iowa, 1976.

Dr. R. P. DiAugustine: Adjunct assistant professor, Department of Medicine, Duke University; invited lecturer at Department of Pathology, Duke University; member of NIEHS Medical Sciences Lectures Committee; invited speaker at American Thoracic Society (Eastern Section), Oct. 1976.

Dr. T. E. Eling: Adjunct assistant professor, Department of Medicine, Duke University; invited speaker at Deer Lodge Conference on clinical pharmacokinetics; invited speaker at Gordon Conference on drug metabolism. Reviewer for DRUG METABOLISM AND DISPOSITION, LIFE SCIENCES, CANCER RESEARCH, JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS.

Dr. L. G. Hart: Adjunct associate professor, Department of Pharmacology, University of North Carolina, lectures presented to medical and graduate students of University of North Carolina; member of NIEHS Safety Committee; NIEHS project director for coordinating efforts in three task areas under the "health effects of the non-nuclear energy technologies" program; associate managing editor (U.S.A.) for CHEMICO-BIOLOGICAL INTERACTIONS; editorial board member of ENVIRONMENTAL HEALTH PERSPECTIVES; chaired symposium on Pesticides in Aquatic Environments, 15th Congress of Entomology, Washington, D. C.

Dr. G.E.R. Hook: Adjunct assistant professor, Department of Medicine, Duke University; co-managing editor and editorial board member of ENVIRONMENTAL HEALTH PERSPECTIVES; editorial board member of CHEMICO-BIOLOGICAL INTERACTIONS.

Dr. R. M. Philpot: Adjunct assistant professor, Department of Entomology, North Carolina State University; member of NIEHS editorial board; editor, CHEMICO-BIOLOGICAL INTERACTIONS.